UNIVERSITAT DE BARCELONA

Facultat de Farmàcia
i Ciències de l'Alimentació

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

Final Degree Project
Degree in Pharmacy

Nucleic Acid Aptamers in Therapeutics and Diagnostics

Alejandro Delgado Quirós

Main field: Biochemistry and Molecular Biology
Secondary fields: Pharmacology and therapeutics,
Pharmaceutical Chemistry

Facultat de Farmàcia i Ciències de l'Alimentació
Universitat de Barcelona
Barcelona, Juny de 2023

DEDICATÒRIA

I would really like to dedicate this final-degree work to all those people who have been with me during this five-year journey. And above all, I dedicate it to myself because even when I was some time on the verge of collapse, I knew how to move forward.

M'agradaria dedicar aquest treball final de grau a totes aquelles persones que han estat amb mi durant aquest viatge de cinc anys. I sobretot, me'l dedico a mi mateix perquè fins i tot quan estava en algun moment prop del col·lapse, he sabut avançar.

AGRAÏMENTS

First, I would like to thank my parents for all the support and freedom they have given me so that I can study what I want and make it possible.
Secondly, I would like to thank the Liats and Starbucks groups for making the stay at University something even more joyful, in particular: Gerard Escarrà, Anna Martinez, Joan Cuixart and Natalia Sánchez, who I will take in my heart forever.
And last, but not least my group of lifelong friends McBlyat and Ana Pacheco, my best friend, for being always by my side.

En primer lloc, vull agrair als meus pares tot el suport i la llibertat que m'han donat perquè pugui estudiar el que vull i fer-ho possible.
En segon lloc, m'agradaria donar les gràcies als grups de Liats i Starbucks per fer encara més alegre l'estada a la Universitat, en particular a: Gerard Escarrà, Anna Martínez, Joan Cuixart i Natàlia Sánchez, els quals portaré al meu cor per sempre.
I per últim, però no menys important, el meu grup d'amics de tota la vida, McBlyat, i a l'Ana Pacheco, la meva millor amiga, per estar sempre al meu costat.

ABBREVIATIONS

ADCs: Antibody-Drug Conjugates.
AMAS: Automated Modular Aptamer Synthesis.
AMC: Age-Associated Macular Degradation.
ApDCs: Aptamer-drug conjugates.
AuNPs: Gold-Nanoparticles.
BPA: Bisphenol A.
CE-SELEX: Capillary Electrophoresis SELEX.
CTCs: Circulating Tumor Cells.
F-: Fluor modification.
HIV: Human Inmunodeficiency Virus.
HTS: High Throughput Sequencing.
LNA: Locked Nucleic Acids.
miRNA: Micro Ribonucleic Acid.
NH_2-: Amino Modification.
Ome: Oxygen-Methyl Modification.
PCR : Polymerase Chain Reaction.
PEG: Polyethylene glycol.
PSMA : Prostate Specific Membrane Antigen.
PS: Phosphorothioate.
PS2: Phosphorodithioate.
PTK7 : Protein Tyrosine Kinase 7.
RNAi : Interference Ribonucleic Acid.
SELEX: Systematic Evolution of Ligands by Exponential enrichment.
shRNA : Short hairpin RNA.
siRNA: Small interfering RNA.
VEGF-A: Vascular endothelial growth factor A.

Abstract

Aptamers are short, single-stranded DNA or RNA molecules that have a high affinity and selectivity for binding to specific target molecules such as proteins, tiny compounds, and even whole cells or viruses. They are frequently referred to as "chemical antibodies" because of their ability to replicate the action of antibodies while offering various advantages over regular antibodies. Aptamers are generated through a process called SELEX, which involves iterative rounds of selection and amplification of the most effective binding sequences from a diverse library of random oligonucleotides. This allows for the creation of aptamers that are highly specific for their target molecule, with affinities that can rival or exceed those of antibodies. They are also less likely to trigger an immune response, which makes them more suitable for diagnostic and therapeutic applications. Although they seem perfect in many ways, those molecules usually require optimization processes to become effective and efficient. Aptamers have a wide range of potential applications, including as diagnostic tools for detecting disease biomarkers, as therapeutic agents for treating a variety of diseases, and as biosensors for detecting toxins and pathogens in food and the environment. Macugen is the only aptamer therapy that has been approved for use and is targeted at dry AMC patients.

Resum

Els aptàmers són molècules curtes d'ADN o ARN monocatenaris que tenen una alta afinitat i selectivitat per unir-se a molècules diana específiques com proteïnes, compostos diminuts i fins i tot cèl·lules senceres o virus. Sovint se'ls coneix com a "anticossos químics" per la seva capacitat de replicar l'acció dels anticossos alhora que ofereixen diversos avantatges sobre els anticossos tradicionals. Els aptàmers es generen mitjançant un procés anomenat SELEX, que implica repetides rondes de selecció i amplificació de les seqüències d'unió més efectives d'una biblioteca diversa d'oligonucleòtids aleatoris. Això permet la creació d'aptàmers molt específics per a la seva molècula objectiu, amb afinitats que poden rivalitzar o fins i tot superar les dels anticossos. També són menys propensos a desencadenar una resposta immune, cosa que els fa més adequats per a aplicacions diagnòstiques i terapèutiques. Encara que semblin idonis en molts aspectes, aquestes molècules requereixen normalment d'un procés d'optimització per esdevenir eficaços i eficients. Els aptàmers tenen una àmplia gamma d'aplicacions potencials, incloent-hi com a eines de diagnòstic per detectar biomarcadors de malalties, com a agents terapèutics per tractar una varietat de patologies, i com a biosensors per detectar toxines i patògens en els aliments i el medi ambient. El Macugen es l'única teràpia que utilitza aptàmers que ha estat aprovada i està dirigida en pacients amb AMC.

Field integration

Starting from the main field of **Biochemistry and Molecular Biology**, aptamers are useful as powerful tools for the study and research of biomolecules. They can specifically bind to a wide number of targets, such as proteins, membrane receptors, or even whole cells. As a result, they can be designed to meet specific objectives, enabling research into biomolecular interactions and understanding biological mechanisms, leading to improvements in the research of difficult molecular interactions and the development of new analytic techniques.

Moving onto **Pharmacology and Therapeutics**, aptamers are aiming to have great potential as therapeutic agents. This is due to their ability to bind to pathologically associated targets such as viral proteins, cancer markers, or bacterial cells with outstanding selectivity and affinity. Furthermore, their capability to interrupt specific protein-protein interactions allows them to research new avenues for therapeutic treatment. Consequently, they are rising as attractive candidates for drug candidates or drug delivery in the medicine field.

Finally, in the **Pharmaceutical Chemistry** realm, aptamers can be useful for studying the structure and function of target molecules, elucidating biological pathways, and developing new drugs. Aptamers can also be integrated into biosensor platforms to detect and quantify target molecules in complex biological samples. They offer rapid and sensitive detection methods for clinical diagnostics and monitoring of therapeutic efficacy. Moreover, aptamers are being designed for the detection of foodborne pathogens, infectious agents, and environmental pollutants.

Their ability to recognize and selectively bind target molecules makes them a promising option for drug development, as diagnostic tools, and as biosensors for endless purposes.

Sustainable Development Goals

Due to the exclusive characteristics of the aptamers, their utility is very deep and can be applied in an infinity of fields. In relation to the Sustainable Development Goals (SDGs), aptamers can meet three of the following:

- **Goal 3 Health and well-being:**

Aptamers are relevant to Goal 3 of promoting health and well-being since these molecules can be adapted to recognize specific biomarkers associated with diseases, making it possible for the early and accurate diagnosis of medical pathologies. This is crucial for fast diagnosis and treatment and is related to target *3.3 By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases.*

Aptamers can also be used as therapeutic agents by specifically targeting proteins or cells linked to disorders, allowing for a more accurate and customized approach to treatment. The purpose of aptamers mainly lies in target *3.d Strengthen the capacity of all countries, in particular developing countries, for early warning, risk reduction and management of national and global health risks.*

- **Goal 6 Clean water and sanitation:**

Aptamers also have implications for Goal 6, which calls for guaranteeing the availability and long-term management of water and food sanitation. They can be used to identify water impurities such as poisons and chemical contaminants. Rapid and sensitive detection systems can be established by creating aptamers that attach especially to certain pollutants, adding to the assurance of water and goods quality and the safety of their consumption. This is critical for preserving human health and the environment, as well as supporting sustainable water management and sanitation practices. Specifically, the aptamers meet point *6.3 By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally.*

- **Goal 12: Responsible consumption and production:**

Regarding goal 12, responsible consumption and production, aptamers can be utilized for producing biosensors that detect chemical residues and pollutants in products, thereby improving food safety and consumer safety. Aptamers can also be employed in place of antibodies in laboratory experiments, reducing the usage of animals in research and the environmental impact of antibody manufacturing. This contributes to the safety of the goods we consume while also promoting responsible and sustainable manufacturing practices, as mentioned in point *12.a Support developing countries to strengthen their scientific and technological capacity to move towards more sustainable patterns of consumption and production.*

To conclude, these three goals are related since these molecules provide novel solutions in the realms of health, environmental protection, and food security, thereby boosting worldwide efforts to achieve sustainable development and improve people's quality of life.

CONTENTS

1. Introduction

We live in a world in constant evolution both socially and technologically. This leads us to want to always seek the best methods and solutions for society. The research for versatile molecular tools has been a constant in scientific and medicinal research in recent years. Regarding the main field of biochemistry and molecular biology, an exploration into the phenomenon of aptamers will be undertaken.

Aptamers are short, single-stranded DNA or RNA molecules that are capable of binding to specific target molecules, such as proteins, small molecules, and even whole cells or viruses, with high affinity and specificity which makes them one of the most promising advances in those sectors (**Fig. 1**).

They are often referred to as "chemical antibodies" due to their ability to mimic the function of antibodies, but with several advantages over traditional antibodies. Aptamers are often compared to antibodies because they can recognize and bind to specific target molecules with high affinity and specificity, similar to how antibodies function in the immune system. However, aptamers have several advantages over antibodies, including their smaller size, greater stability, and ease of synthesis and modification. Additionally, aptamers can be selected to bind to targets that are difficult or impossible to target with antibodies, such as small molecules or non-immunogenic targets (1).

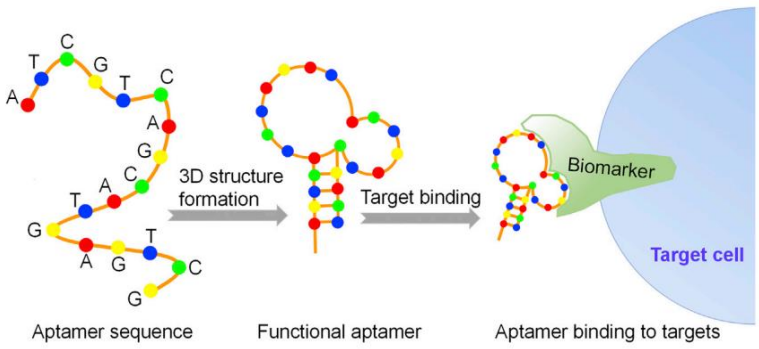

Fig. 1. The evolution of the aptamer from the sequence into functional form and target binding (2).

Aptamers are typically selected from a large pool of random oligonucleotides or peptides through a process known as systematic evolution of ligands by exponential enrichment (**SELEX**). During **SELEX**, the library of oligonucleotides or peptides is subjected to multiple rounds of selection, where the molecules that bind most strongly to the target are retained and amplified, while those that bind weakly or not at all are removed. This process is repeated several times, resulting in the isolation of a pool of aptamers that bind to the target with high affinity and specificity (3).

Not all aptamers' capabilities are beneficial, they also have two main weaknesses: low resistance to nucleases and short circulation time due to high renal clearance. This corresponds to a significantly shorter in vivo time.

Aptamers, however, offer a great range of potential applications, including as therapeutic agents for treating several diseases, diagnostic tools for identifying disease biomarkers, and biosensors for recognizing toxins and pathogens in food and the environment.

2. Objectives

The purpose of this TFG is to do bibliographic research about the aptamers. The aim of this work is to enter in detail about a few objectives:

- Present the molecular biology and chemistry of aptamers.
- Show how the aptamers are created.
- Expose the advantages and drawbacks of aptamers.
- Compare them to the current antibodies.
- Explain the different applications of aptamers in various fields.
- Describe in detail an example of a commercialized aptamer (Macugen).

3. Materials and methods

This project belongs to the category of bibliographic research. Most of the information has been obtained from different sources of information, in particular, from scientific articles. Access to them has come from different databases, such as Scopus, Elsevier, and PubMed, among others. To get more detailed information about my goals, I have used advanced search methods using keywords. An example of such searches could be "Aptamers", "SELEX", "Aptamers AND Therapeutics" and "Aptamers AND Biosensors". To obtain the most relevant information, I have used the most frequently cited filter to be able to view more relevant articles. Besides, I have also kept in mind the most recent articles to be able to give the most updated information possible.

4. Results and discussion

4.1 SELEX method for aptamer generation

The **SELEX** (systematic evolution of ligands by exponential enrichment) method is a technique, that was developed in the 1990s by Tuerk and Gold (4), and Elleingtong and Szostak (5), used to generate aptamers. The **SELEX** process typically begins by synthesizing or buying a library of random **RNA** or **DNA** oligonucleotides, usually consisting of around 10^{15} different sequences. These sequences are compound by a random central region, up to 80 nucleotides, and specific primers at 5' and 3' regions that will be used later for the **PCR** procedure (3).

RNA libraries were traditionally the most preferred choice for aptamer creation because it was thought that **RNA** had the unique potential to take on functional structures. On the other hand, it demands the addition of a reverse transcription step to each **SELEX** cycle. This opens the door to transcriptional mistakes, which grow more severe with longer **RNA** sequences of roughly 100 nucleotides and are also more susceptible to misfolding.

These drawbacks raised the appeal of **DNA** libraries. In addition to skipping the reverse transcription step, **DNA** oligonucleotides have superior chemical and biological stability and are easier to produce (**Fig. 2**). Although, in terms of enzymatic activity, **RNA** does not always provide a competitive advantage. Deoxyribozymes do not exist in nature, but they can be produced in vitro from longer sequences without the limitations associated with **RNA**. **DNA** libraries eventually surpassed **RNA** libraries in popularity, and they are now the most widely used in literature and on the market (6).

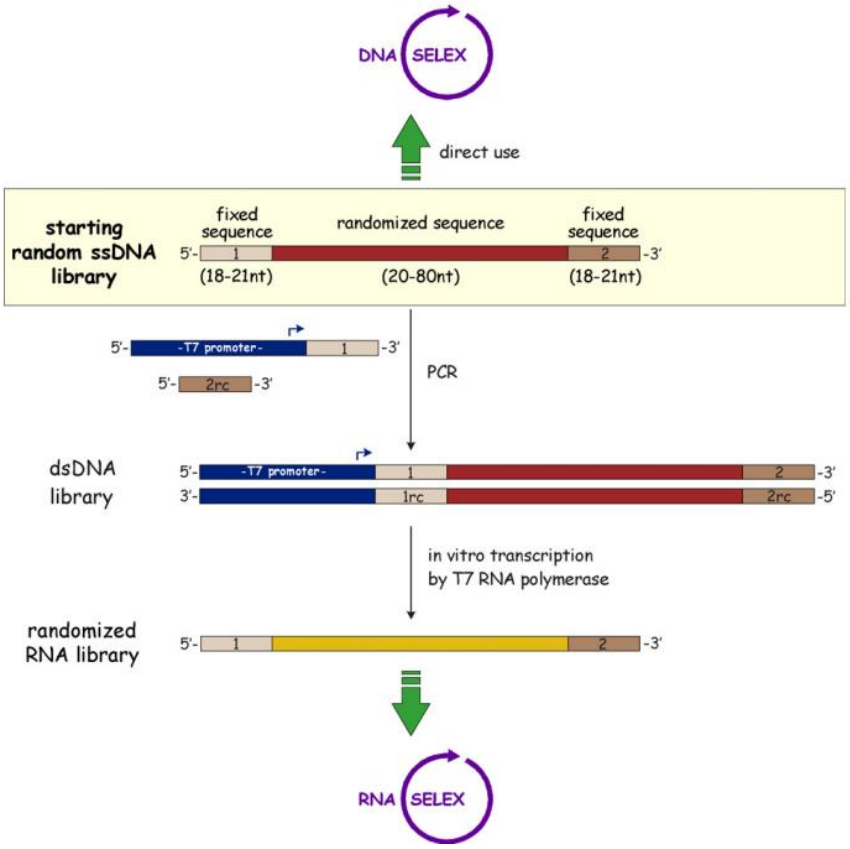

Fig. 2. Two different SELEX starting procedures from a random ssDNA library (7).

The chosen library is then exposed to the target molecule, which could be a protein, a small molecule, or a whole cell or virus. The oligonucleotides that bind to the target molecule are then separated from those that do not bind using a variety of methods, such as affinity chromatography, magnetic beads, or fluorescence-based assays. The reason for binding to the target is caused by structural compatibility, aromatic ring stacking, electrostatic and van der Waals interactions, hydrogen bonding, or a combination of these factors. These facts allow them to fit into a large mix of targets, from a receptor to an entire cell (7).

The selected oligonucleotides are then amplified using polymerase chain reaction (**PCR**), which generates large amounts of copies of the selected sequences. The amplified sequences are then used to generate a new library for the next round of selection. This process is repeated for several rounds, typically ranging from 5 to 15, depending on the desired level of affinity and specificity.

As the **SELEX** process progresses, the oligonucleotide library becomes enriched for sequences that bind to the target molecule with higher affinity and specificity. This is because the sequences that do not bind as well to the target molecule are progressively eliminated with each round of selection, while the sequences that bind more strongly are preferentially amplified and carried forward to the next round.

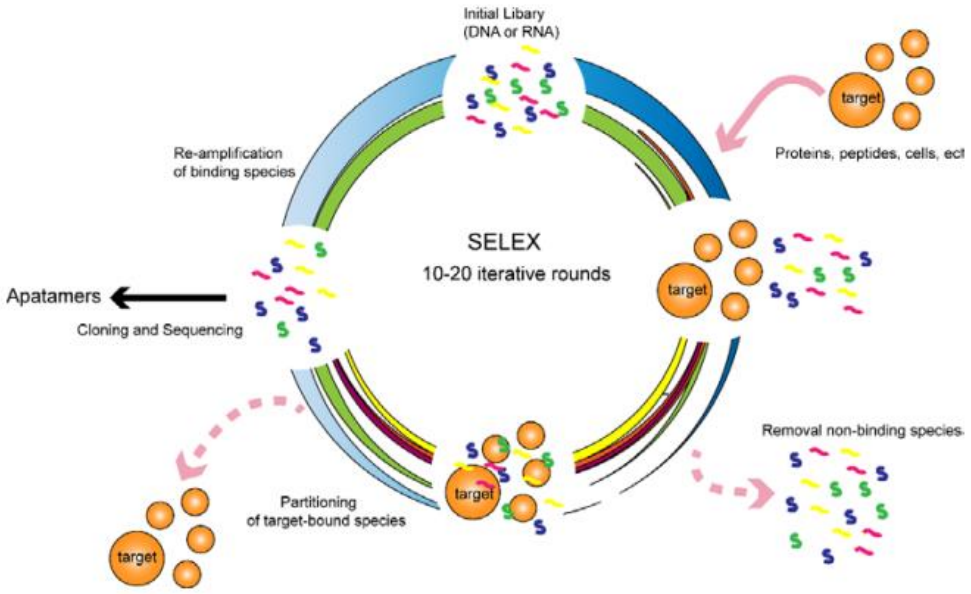

Fig. 3. SELEX procedure scheme (8).

Finally, the enriched oligonucleotides are sequenced and analyzed to identify the most effective binding sequences. These sequences can then be synthesized and tested to confirm their binding properties and specificity. Once confirmed, the aptamers can be used for a variety of applications, such as diagnostic assays, therapeutic agents, and biosensors (**Fig. 3**).

4.1.1 Different SELEX methods

Obtaining specific aptamer candidates might take weeks to months, and hit rates are low. As a result, obtaining high-quality aptamers against relevant targets remains a bottleneck. Several updated **SELEX** techniques have been implemented to reduce selection time and increase hit rates.

In general, the traditional **SELEX** approach, which is more expensive and time-consuming, requires more than 15 cycles to obtain aptamers. **Capillary Electrophoresis SELEX (CE-SELEX)** was created in 2004 as a modified **SELEX** technology (9). **CE-SELEX** separates target-confined sequences from unbound sequences using electrophoretic mobility differences, which is a highly efficient separation approach. This method allows for the selection of aptamer candidates with strong affinity while lowering the number of selection rounds from almost 20 in standard **SELEX** to 1-4. Several aptamers have been successfully selected using **CE-SELEX** to date (8).

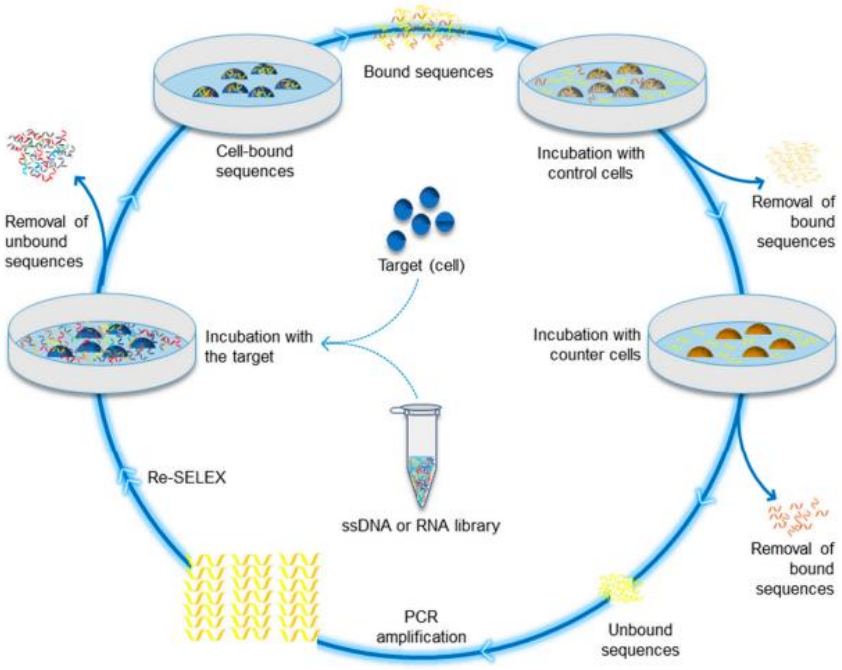

Fig. 4. CELL SELEX schematic procedure. Starting with a DNA or RNA library, followed by the incubation of the target with the library and, finally the amplification of the selected strands (1).

The classic **SELEX** method has obtained many aptamers targeting purified proteins in vitro, but most of these aptamers may not be able to bind to the same proteins in cells at physiological circumstances. **Cell-SELEX** targets complete live cells, increasing the chances of the chosen aptamer being employed directly for diagnostic and therapeutic applications, as shown in **Fig. 4**. **Cell-SELEX** offers several advantages over in vitro SELEX, like the fact that the molecular targets on the cell surface are in their native conformation; the aptamers obtained represent the final results; no protein purification or prior knowledge of the molecular targets on the cell surface is required before selection, and also this method could be used to discover new biomarkers (8).

In summary, the different **SELEX** methods are a powerful technique for generating aptamers with high affinity and specificity for a wide range of target molecules. The iterative process of selection and amplification allows for the isolation of the most effective binding sequences, and the resulting aptamers have an extensive range of potential applications in various fields (1). In **Table 1**, a few SELEX modification procedures are shown.

SELEX Type	Principle	Aim	Application/Result	Year
Spiegelmer®	Selection of D-oligonucleotides against a mirrored target. Then, the D-oligonucleotides will be synthesized as L-oligonucleotides that can bind to unmirrored target	Increase aptamer stability against nucleases	Identified an endonuclease-stable L-RNAaptamer that binds to D-adenosine	1996
Chimeric SELEX	Fuses pre-selected aptamers of different targets together to form a combined small library followed by applying a dual selection pressure to select aptamers that can bind to multiple targets	Generate dual-function aptamers	Generated an engineered dual-function aptamer capable of testing/binding to two distinct features/targets	1998
SOMAmers	▪ Increases the physicochemical diversity of the library through incorporating chemically modified nucleotides ▪ Transforms the protein concentration signature	▪ Select high-affinity aptamer of very slow off-rate ▪ Detect hundreds of proteins (large-scale proteome analysis) in small sample volumes	▪ Provided a new tool for screening biomarkers ▪ Identified 813 proteins with high sensitivity (low detection limit of ~1 pM)	2010
HTS-SELEX	One round of positive selection followed by HTS and informatic analysis	▪ Produce high-affinity aptamer in one positive selection round ▪ Does not require tedious work	Identified aptamers that can bind to thrombin in nM range	2011

Table 1. Different SELEX modified procedures (10)(11)(12)(13).

4.2 Aptamer application fields

4.2.1 Therapeutic applications

Starting in the therapeutics field, aptamers have several advantages over traditional small molecule drugs and antibodies, which make them a promising class of molecules.

4.2.1.1 Aptamers vs Antibodies

Aptamers and antibodies are both molecules that can bind specifically to a target molecule, but they differ in several important ways (14) (3):

- **Size and flexibility:** Aptamers are smaller and more flexible than antibodies, which allows them to access binding sites that may be inaccessible to larger molecules (**Fig. 5**). Aptamers have a smaller molecular weight (5-15 kDa) than antibodies (150-1000 kDa), which allows them to have fewer steric limitations and significantly faster diffusion rates, allowing them to more frequently encounter biomembrane structures and more quickly diffuse into binding sites. Clinical investigations have revealed that, as compared to antibodies, the human body can tolerate micromolar doses of oligonucleotides and greater penetration of aptamers into the tumor. Antibodies are much larger and less flexible, which can limit their ability to bind to certain targets.

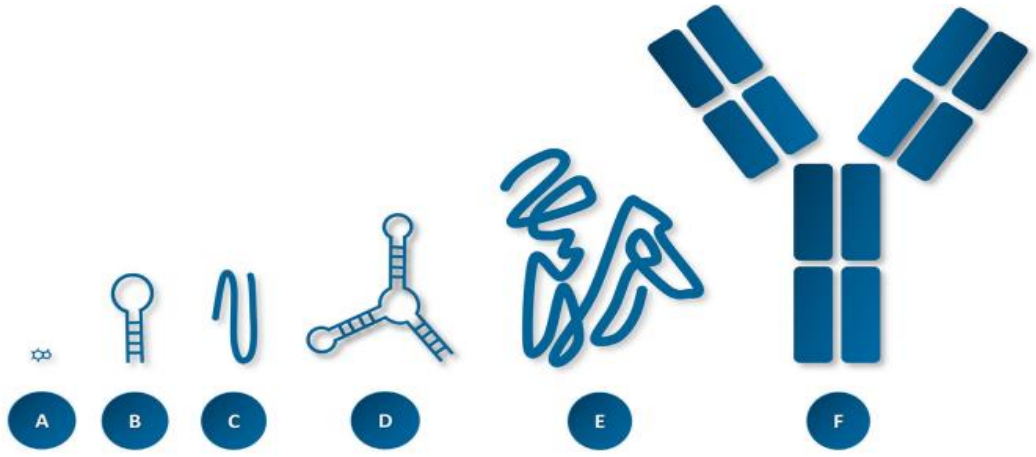

Fig. 5. Molecules comparison. A) Caffeine molecule, 0.2 kDa; (B) 50mer oligonucleotide, 16 kDa; (C) human insulin, 6 kDa; (D) 100mer oligonucleotide, 32 kDa; (E) human serum albumin, 66 kDa; (F) IgG1-antibody, 150 kDa (15).

- **Production:** Aptamers are generated through a chemical synthesis process, whereas antibodies are typically produced using live cells or animals. Aptamers are simple to chemically synthesize and modify, and they are appropriate for large-scale synthesis using solid-phase technology with no batch-to-batch variations. Organic processes have been used to alter antibodies, but they have the disadvantage of being difficult to prepare and having an unpredictable conjugation number and site. To put several functional particles onto one aptamer at predetermined places and loading capacity at a reasonable cost, automated modular synthesis and phosphonamidite chemistry have been built. These techniques offer numerous benefits, including simplicity of scaling, ease of operation, great efficiency, and a low price. This makes the production of aptamers faster and more scalable than the production of antibodies.
- **Specificity and affinity:** Aptamers can be generated to bind to a great range of target molecules, including small molecules, proteins, and even whole cells or viruses, with high specificity and affinity (**Fig. 6**). Antibodies can also be generated with high specificity and affinity, but they may require additional modifications or engineering to achieve this.

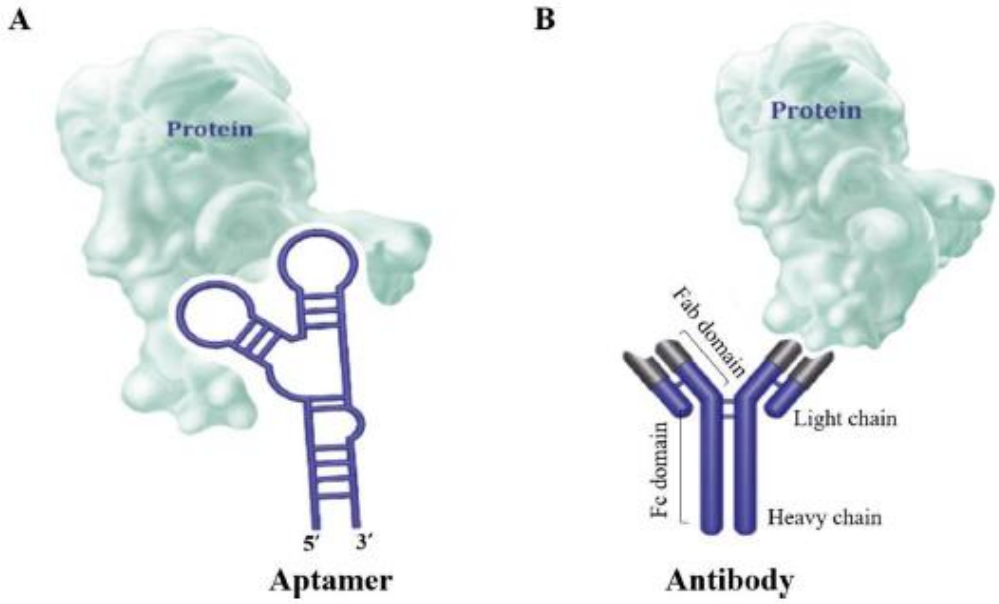

Fig. 6. Aptamer and antibody reaching their target (14).

- **Stability:** Under the right conditions, aptamers can be stable and resistant to degradation; nevertheless, antibodies are more durable due to their complex structure and the existence of disulphide bonds. To strengthen their stability, aptamers can be built with hairpin structures or with specific chemical modifications at the ends.
- **Immunogenicity:** Antibodies can be immunogenic, meaning that they can trigger an immune response in the body. Aptamers, on the other hand, are less likely to trigger an immune response, which makes them more suitable for diagnostic and therapeutic applications.
- **Cost:** Aptamers are generally less expensive to produce than antibodies, as they can be synthesized chemically. Antibodies require the use of live cells or animals, which can be costly and time-consuming. Additionally, aptamers can be easily synthesized and modified which reduces the cost and time of production and also facilitates large-scale production.

As shown in **Table 2**, compared to antibodies, aptamers have two weak points: **nuclease hydrolysis** and a poor time in the organism because they usually have **fast clearance** through renal filtration. Both weak points lead us to have a really short in vivo half-life. Further, different techniques and strategies that can be applied to alleviate these inconveniences will be addressed.

	Aptamer	Antibodies
Molecular weight	Small (~12–30 kDa)	Relatively big (~150–180 kDa)
Secondary structures	Various structures: hairpin, loop, G-quadruplex, *etc*	β-sheets
Generation time	Few hours to months	Several months (~six months)
Batches variations	Low	High
Immunogenicity	Low	High
Minimal target size	Targets small sizes ~60 Da	~600 Da
Targets	Wide range of targets	Immunogenic molecules
Shelf life	Long	Short
Allowed chemical modifications	Various modifications	Limited modifications
Nuclease degradation	Sensitive	Resistant
In vivo half-life	Short (~20 min)	Long (~one month)
Stability	Very stable	Sensitive to temperature and pH changes
Cost	Lower	Higher

Table 2. Physicochemical properties of aptamers and antibodies (8).

4.2.1.2 Aptamers as therapeutic agents

Aptamers can be used in a variety of therapeutic applications, including **cancer therapy** (2), infectious **disease treatment**, and **targeted drug delivery** (3).

One approach is to use aptamers as inhibitors of disease-related proteins, such as enzymes or receptors. For example, an aptamer that specifically binds to a cancer-related protein could be used to block its activity and inhibit tumor growth. This approach has been demonstrated in several preclinical and clinical studies, where aptamers have been shown to effectively inhibit disease-related proteins in vitro and in vivo (8). There is now just one aptamer-based treatment on the market, **Macugen (Pegaptanib)**. It is used to treat age-associated macular degeneration (**AMC**), the treatment of which will be discussed later.

As **drug delivers**, aptamers are conjugated to a therapeutic agent, such as a chemotherapeutic drug or a **siRNA**, and delivered specifically to the diseased tissue or cell. This approach has several advantages over traditional drug delivery methods, including increased selectivity, reduced toxicity, and improved efficacy. Aptamers have been successfully used to deliver drugs to cancer cells and **HIV**-infected cells in preclinical studies. They can be classified into three different categories (3):

- **Aptamer-Drug Conjugates (ApDCs)**: One of the most common limitations of chemotherapy is the low selectivity of the drugs, which usually produces several side effects. Different research groups have developed conjugated aptamers that bind or deliver the drug to the target more effectively than naked drugs or even show themselves superior compared to antibody-drug conjugates (**ADCs**).

  Another advantage is that aptamers can be examined in several days instead of the laborious in vivo screening process used in antibody discovery during the production of the targeted ligand. Furthermore, aptamers can target a wide range of molecules, including those with low immunogenicity that are not recognized by antibodies. Additionally, aptamers can be chemically generated like conventional chemical entities throughout the preparation process under good manufacturing practice (**GMP**). The utilization of a series of cell colonies and living animals in the manufacture of antibodies can increase contamination and result in substantial batch-to-batch variance.

  Moreover, **ADCs** may trigger the body to generate antibodies against them during the application process, limiting their therapeutic efficacy. At the moment, aptamer preparation basically follows the same procedures as antibody development. As an alternative, automated modular aptamer synthesis (**AMAS**) has various advantages over traditional synthesis methods. It enables the simultaneous synthesis of numerous aptamer sequences, considerably improving throughput. It also decreases human error, enhances reproducibility, and allows for the exploration of huge aptamer libraries for screening (16).

- **Aptamer–macromolecule conjugated systems:** In this category, there are a lot of candidates that can be used in therapy, like RNAs, peptides, or even liposomes. Starting with **RNAi** therapy is an excellent technique for illness treatment. The lack of selectivity and specificity for this therapy, on the other hand, impedes the successful translation of its broad clinical application. Aptamers linked to microRNA (**miRNA**), small interfering RNA (**siRNA**), and short hairpin RNA (**shRNA**) have thus been widely investigated for targeted delivery of these functional **RNAi** into the target area (8).

Different kinds of conjugated RNAs, **RNAi**, **miRNA** and **shRNA** for different target locations are being developed (17)(11).

For example, the very first developed **siRNA** aptamer chimera (11), was a linking between a targeted aptamer to a prostate-specific membrane antigen (**PSMA**) and a therapeutic **siRNA** against two overexpressed genes in many cancer cells (**Fig. 7**). The chimeras precisely target prostate cancer cells expressing **PSMA** and silence the target gene of **siRNA**, resulting in targeted suppression of tumor development in a prostate cancer model.

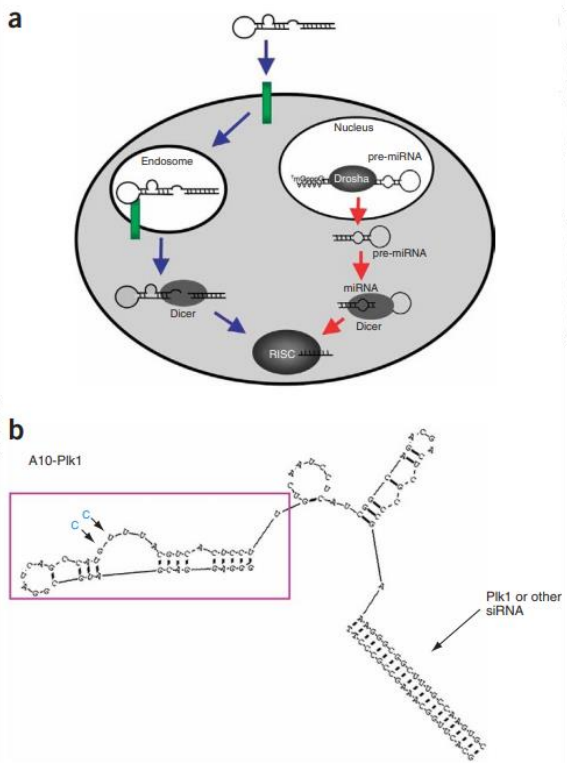

Fig. 7. Proposed mechanism of action and secondary structures of the chimeras. (a) The chimera binds to the cell surface receptor and follows the RNAi pathway. (b) Secondary structure of the chimera (called A10). PSMA binding region is pink outlined (11).

Talking about immunotherapies field, most of them have several problems arriving at the target and penetrating the cell membranes of the target tumors. These problems can be solved with aptamers due to their biocompatibility and minimal immunotoxicity (18). In this field, most of the aptamers in research are vaccines targeted for the delivery of cytokines or peptide antigens. Several studies have proven effective and specific techniques for the delivery of peptide antigens toward antigen-presenting cells **APCs** (19).

Furthermore, liposomes can also be used in conjugation with aptamers as a targeted therapy due to their dual hydrophobic and hydrophilic properties, which allow them to encapsulate either hydrophilic or hydrophobic medicines (8).

Also, by modifying the surface of the liposome with high-molecular-weight **PEG** molecules, results in higher plasma half-lives and effective accumulation at the target sites (**Fig. 8**).

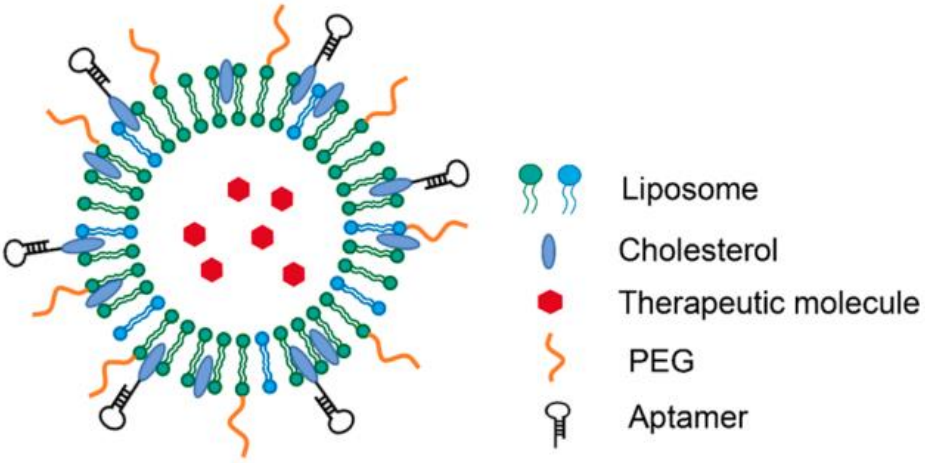

Fig. 8. Schematic illustration of an aptamer-liposome conjugated (3).

- **Aptamer-Nanomaterial**: Because of their intrinsic unique physicochemical attributes, nanomaterials have various advantages, including an ultra-small size, a large surface effect, and an excellent absorption rate. They are promising drug delivery carriers because of their comparatively wide surface area, which allows them to include several targeting ligands or additional therapeutic agents (8). In this type of therapy, the selected aptamer is attached to an inert moiety (**Fig. 9**). The most used material in this type of research is gold because those nanoparticles (**AuNPs**) have numerous advantages, including biocompatibility, non-toxicity, simple modification, and exceptional stability (20).

  Luo et al. (20) created a smart aptamer-based medication delivery device. The **DNA** aptamer was functionalized onto the surface of **AuNPs** as a targeting moiety against protein tyrosine kinase 7 (**PTK7**). In addition, **hpDNA** with a repeating CGATCG deoxyribonucleic acid unit was formed on the **AuNP** surface, which was employed as a delivery vehicle for the chemotherapeutic medication doxorubicin.

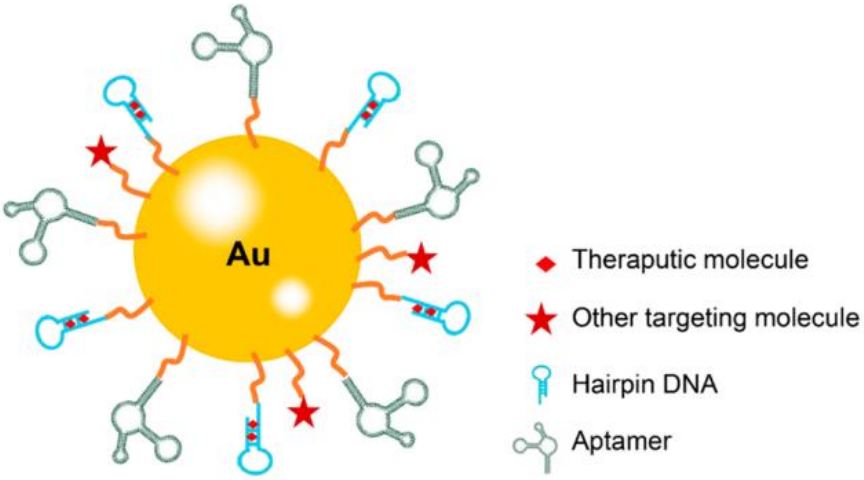

Fig. 9. Diagram based on aptamer-conjugated gold particle AuNP. Usually, AuNPs also carry hpDNA as a drug cargo (3).

4.2.1.3 Aptamers as diagnostic tools

Another of the advantages that aptamers offer us is their specificity for binding to disease biomarkers. This capacity can be used to develop sensitive and specific diagnostic assays for early disease detection. Song et al. (21), for example, designed a microfluidic chip with multivalent aptamer nanospheres. When compared to the monovalent aptamer-modified chip, the capture efficiency of circulating tumor cells (**CTCs**) was increased by employing high-density multivalent aptamers and a micropillar structure (**Fig. 10**)**.**

In another study, Zhang et al. (22) used aptamers to develop a method for quick capture and non-destructive release of extracellular vesicles. In terms of isolating targets, aptamers have a significant advantage over antibodies in terms of traceless or label-free release. By adding complementary DNA strands, captured targets can be eluted. This skill is required for researchers to retrieve pure samples as well as for further processes like culture and therapy. In addition, aptamers can be used to monitor disease progression and treatment response, providing valuable information for disease management.

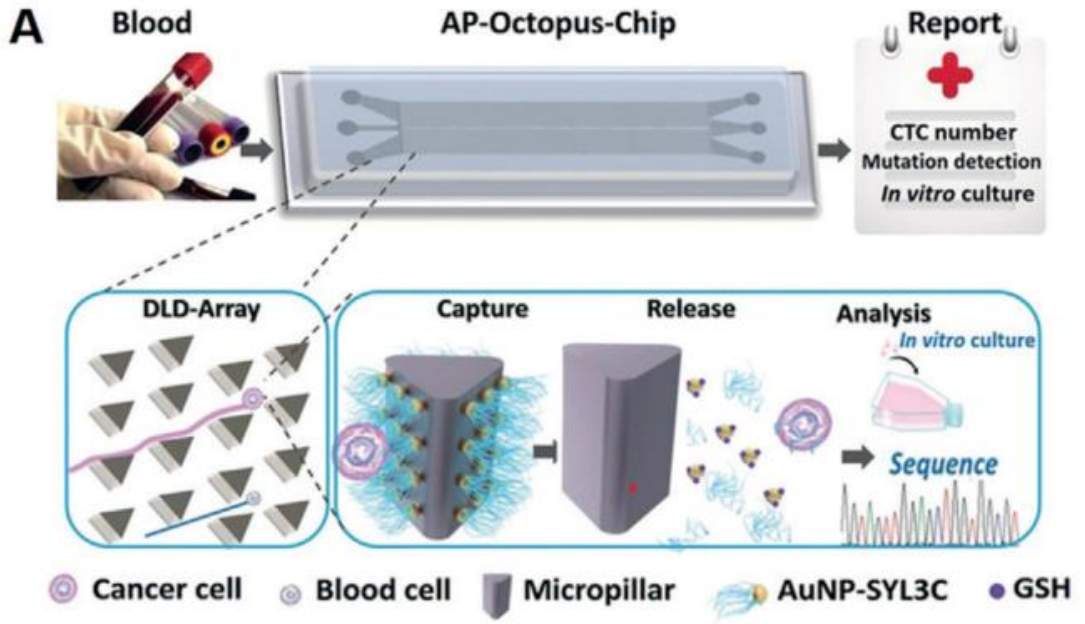

Fig. 10. Circulating Tumor Cells (CTCs) captured on a chip using aptamer-modified AuNPs. CTCs will be constantly colliding with the micropillars, while other blood cells with low interaction can flow normally. The multivalent aptamers have an improved affinity, leading to a better CTCs capture. Then can be easily eluded and analysed. (21).

4.2.2 Aptamer as biosensors

Aptamers can also be used as biosensors, they offer several advantages, including high sensitivity and selectivity, low cost, and easy integration with readout systems. Aptamer biosensors have an extensive range of potential applications, including medical diagnosis, environmental monitoring, and food safety testing.

With current demand for food supplies, the necessity for strong food laws, and safety measures have risen to the top of the list of health issues. Unsafe food is strongly connected to an illness. An estimated 2 million deaths occur each year, including children. Food tainted with infections, microorganisms, or chemical compounds causes more than 200 diseases (24). New challenges to food safety include, among other things, changes in manufacturing and distribution techniques, altered food habits, emerging pathogens, and increased contamination owing to increasing travel and trade.

These problems could be solved with different types of aptamer biosensors, usually called **aptasensors** (24). Aptasensors are basically composed of nanomaterials like gold or carbon fiber and aptamers, which are the recognition elements. For these materials, a huge variety of signal transducers have been used in aptasensors for food safety.

Optical aptasensors like colorimetrics or fluorimetrics are currently being used due to their simplicity, rapidity, and sensitivity.

For example, Li et al. (24) found **Hg^{2+}** in water samples using **AuNps** with colorimetric nanosensors. **AuNps** that are often used don't need surface modifications, they don't need to be labeled, the signal can be easily watched because it is a fast color transformation, and the experiment can be done in an aqueous solution. Aptamers specific to mercury are rich in thymine bases, and mercury tends to create thymine-mercury ions with its aptamer. This interaction is more particular than the one between aptamers and **AuNPs** and are left unprotected in solution in the presence of mercury ions. As a result, the presence of NaCl in the solution can screen the electrostatic repulsive forces surrounding **AuNPs**, making them vulnerable to aggregation and colouring the solution purple.

Several antibiotics, pesticides, heavy metals, toxins and microbial cells have also been detected in food and water samples using colorimetric aptasensing technology (**Fig. 11**).

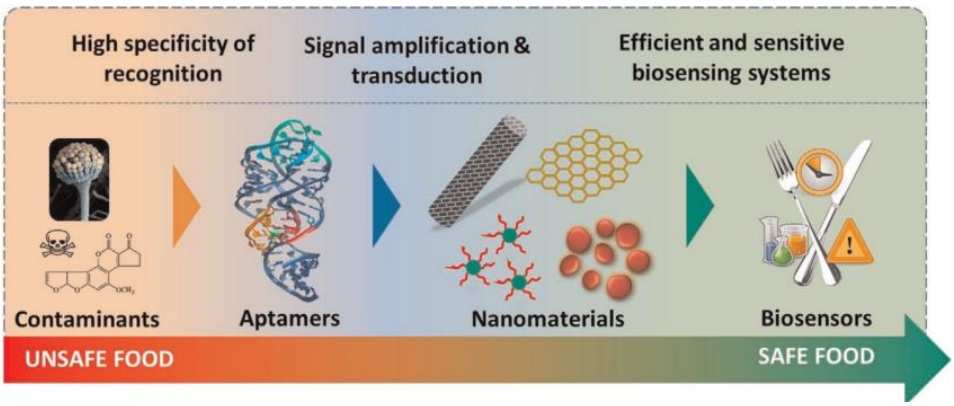

Fig. 11. A Schematic representation of how aptasensors can be used for obtaining safe food (24).

Another type of aptasensors are **electrochemical biosensors**. These devices are the most used because of their selectivity and real time applications. In an electrochemical sensor, the aptamer is immobilized onto the surface of an electrode (25), which serves as the transducer for converting the binding event into an electrical signal. The aptamer undergoes a conformational change upon binding to its target analyte, which leads to a change in the electrochemical properties of the electrode surface. Recently, as an example of food health, an electrochemical aptasensor for bisphenol A (**BPA**) measurement in milk products based on gold nanoparticles dotted with graphene nanocomposite films modified with glassy carbon electrodes was developed (26).

Regardless of method, the creation of these biosensors follows a general line of creation (14):

1. **Attachment of aptamers to a surface**: After selecting specific aptamers, they must be immobilized on a surface for use as a biosensor. Aptamers can be attached to several surfaces, including gold nanoparticles, glass slides, and microbeads.
2. **Detection of target molecules:** When the target molecule is present, it attaches precisely to the surface-immobilized aptamer. Depending on the biosensor and nanomaterial utilized, this binding event can be detected in a variety of ways.
3. **Signal amplification and readout**: Signal amplification techniques are sometimes employed to improve detection sensitivity. Some nanoparticles can be employed to enhance the signal by attaching to the target molecule and boosting scattering strength. After the signal is amplified, it can be detected by a readout system, such as fluorescence, colorimetry, or electrochemistry.

A few examples of aptasensors for different contaminants like pesticides or heavy metals are shown in **Table 3**.

Target compound	Biosensing principle	Nanoparticles	Detection limit (LOD)
Pesticides and insecticides			
Isocarbophos	Nanoparticles as Raman labels in SERS	Ag dendrites	3.4 μM
Omethoate			24 μM
Antibiotics, drugs and their residues			
Oxytetracycline	Non-crosslinking aggregation	AuNPs	25 nM
Kanamycin, kanamycin B, tobramycin		AuNPs	25 nM
Heavy metals			
Mercury (Hg^{2+}) ions	Non-crosslinking aggregation	AuNPs	0.6 nM
Arsenic (As^{3+}) ions		AuNPs	5.3 ppb
Microbial cells			
Salmonella typhimurium	Reduction of silver to produce dark colour	AuNPs	7 cfu/mL
Escherichia coli Listeria mono-cytogenes Salmonella enterica	Chromato-graphic strip based	AuNPs QDs	3000–6000 cells/test (AuNPs) 300–600 cells/test (QDs)
Other analytes			
BPA	Electrochemical aptasensor	AuNP dotted graphene nano-composite film	5 nM

Table 3. Different aptasensors for food and health applications (23) (24) (27) (26) (28) (29) (30) (31) (32).

4.3 Aptamer optimization

Although nucleic acid aptamers offer many advantages, their intrinsic physicochemical properties, such as short half-lives, susceptibility to nuclease degradation, and fast renal filtration excretion, have hampered their in vivo therapeutic effectiveness (15).

To begin, aptamers are made up of nucleic acid sequences, either **DNA** or **RNA**, that are naturally sensitive to degradation by nucleases, which are enzymes that break down nucleic acids. Nucleic acids, unlike more complex protein structures, are likely to be degraded by nucleases, limiting their stability in biological contexts.

Furthermore, the single-chain structure of aptamers makes them more susceptible to nuclease action. Nuclease enzymes can easily recognize and bind to single-chain aptamers, leading them to be degraded quickly. When compared to proteins and antibodies, their lack of stable three-dimensional structure reduces their resistance to enzymatic degradation.

The rapid renal filtration is attributable to the aptamer tiny size and physicochemical features that facilitate their removal through the kidneys. Because of their small size, they can pass past the pores of the renal glomerulus. Besides, the kidneys tend to retain positively charged molecules; some aptamers may contain a negative charge, which aids in their filtration and ulterior excretion in the urine. Modifications and conjugations of

aptamers have been devised to overcome limitations in the implementation of aptamer-based therapies.

In order to deal with these drawbacks, various methods have been tried, focused on the modification of the chains of nucleic acids or the conjugation of the same aptamers to extend their in vivo lifetime. Most of them are included in the SELEX procedure, and by making a few modifications, the conventional SELEX method can be really improved (3).

4.3.1 Aptamer molecule modification

One of the first variations that can be made is to add **modified nucleotides, sugar rings, or even phosphates** (3). The advantage of these modifications arises from changing dT bases for dU bases modified at the 5' position of the heterocyclic base. With a few hydrophobic replacements at this position, like incorporating a benzyl or an indole, a great number of aptamers have been created for different targets that were impossible at the beginning. This achievement has been made by the **SomaLogic** company, which has named their aptamers **SOMAmers**. At the moment, they have identified **SOMAmers** for more than 5000 proteins (12).

Modifications on the sugar ring, including (**F, NH_2, OMe and LNA**) have been introduced by adding unnatural nucleotides by using the mutational **T7RNA** polymerase. Phosphate links can also be modified ty stabilize the chains of nucleotides by replacing the conventional phosphate with sulfur containing phosphate ester bonds like phosphorothioate (**PS**) and phopsphorodithiorate (**PS2**) bonds, as shown in **Fig. 12**.

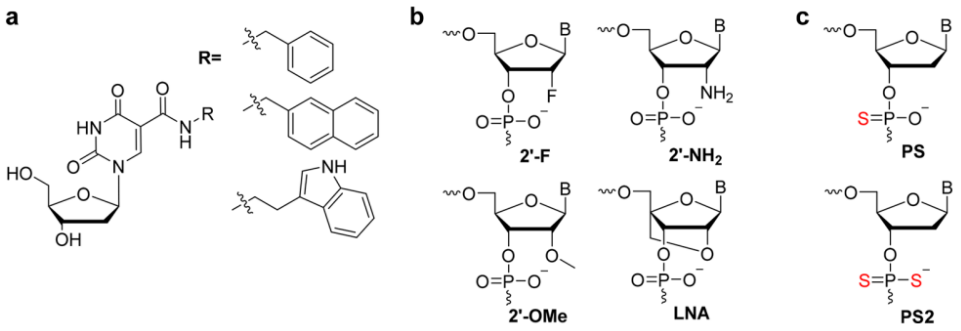

Fig. 12. Modified nucleotides for SELEX procedure. (a) Modifications in the selection of SOMAmers (benzyl, napthyl and indole). (b) Sugar ring modifications, including: F, NH_2, OMe and LNA. (c) Increased stability aptamers garnered by (PS) and (PS2) linkages (3).

Aptamers conjugated with a large inert molecule can be developed as well, such as polyethylene glycol (**Fig. 13**). **PEG**, liposomes, or proteins are an effective way to prevent the rapid elimination of aptamers and prolong their time in serum. Of all the molecules mentioned above, **PEG** is the one that has most widely demonstrated an improvement in half-life time in aptamers that are in development.

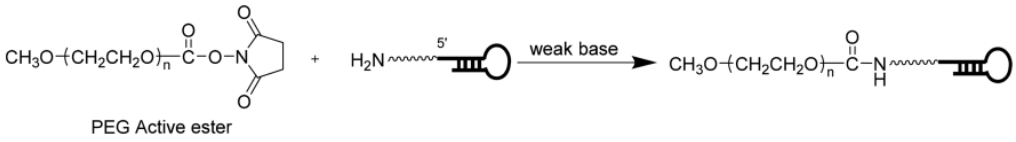

Fig. 13. Addition of a PEG molecule into the 5' terminal of an aptamer (3).

The size of normal aptamers is 6 to 30 kDa and their diameter is usually less than 5nm. When **PEG-conjugated** aptamers are built, their weight grows above 30 to 50 kDa, which is the limit of renal filtration. The union of equal aptamers to form a larger one is another method to increase the size of the aptamers and to increase their retention in blood circulation.

In **Fig. 14**, are shown a set of different modifications that are commonly used in aptamer creation. Some modifications are phosphodiester linkage modifications, sugar ring modifications, and base modifications. The capping of the **3'-end** with inverted thymidine and **PEGylation** has also been frequently used in chemical aptamers for

clinical therapeutic development. Novel approaches exploiting endogenous serum albumin lifetime, such as circular aptamers and lipid-conjugated oligonucleotides, are also disclosed.

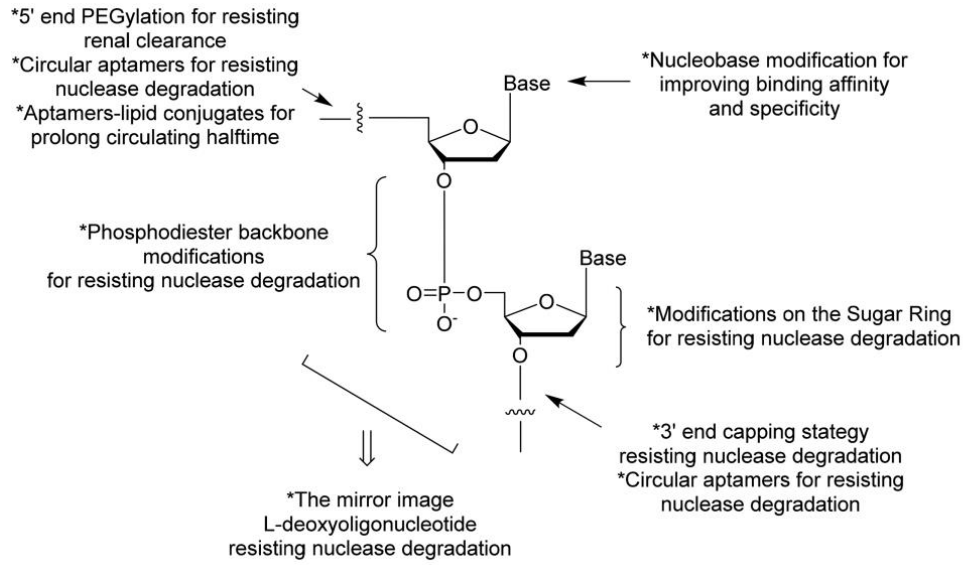

Fig. 14. Most commonly used strategies in the modification of aptamer oligonucleotides and their purposes (3).

All these variations have been shown to be effective in increasing the stability and resistance to nucleases and prolonging serum half-life. Even so, many trials still must be done since these modifications could produce some toxicity or specific immune responses.

4.3.2 SELEX modifications

Another variation is the **Mirror Image Aptamers,** called **Spiegelmers** (10). These aptamers are levorotatory nucleotides, unlike currents, which are dextrorotatory. They exhibit outstanding stability in serum. As shown in **Fig 15**, to obtain this kind of aptamers the synthesis begins with the mirror version of the target. Then natural D-nucleotides are used during **SELEX** against those mirror image targets. The incorporation of natural nucleotides is necessary because the enzymes that work in the hole process are stereoselective. Finally, the obtained sequences are chemically synthesized using enantiomeric L-ribonucleotides (**Fig. 15**).

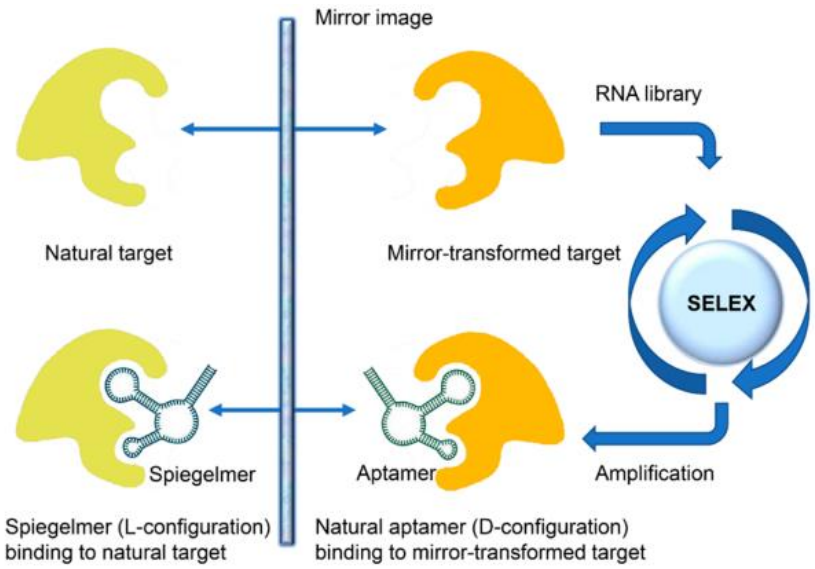

Fig. 15. Illustration of how RNA-Spiegelmer is generated (3).

The **NOXXON** company has many different patents for the preparation and discovery of **Spiegelmers**. They currently have three Spieglemers developed for therapeutic application that have shown great effectiveness in phase 2 studies.

A new type of **SELEX** called **microfluidic SELEX with high-throughput sequencing (HTS)** can also be used. This approach combines the power of **SELEX** with the advantages of microfluidics, such as precise control of fluid flow and the ability to perform reactions on a small scale. While the traditional **SELEX** needs about more than 10 rounds to obtain a valid number of candidates, the microfluidic technique allows us to have detailed control

over a small number of targets in a few rounds. Thanks to this technique, the bias of the usual **SELEX** technique can be reduced, which also increases reproducibility (33).

Inside the microfluidic machines, there are ferromagnetic items that are used to trap paramagnetic beads with the target on them. Once the target is attached, a washing buffer is passed, and up next, the beds will be strictly washed. After this procedure, most unbound oligonucleotides will be eliminated. However, the number of sequences that can be obtained is small and may not be the best binders for the initial pool. To deal with this problem, **HTS** can be added to the procedure. Many scientists agreed that aptamers with the greatest affinity are often those that bind first to the target at the first round of the cycles (13), and by using **HTS**, it is possible to find the highest-fold enrichment in the first cycles of the selection.

Considering an aptamer's function is determined by its structure, most aptamers may experience changes in characteristics as a result of the post-SELEX alteration, affecting binding affinity. As a result, the eventual function of altered aptamers must be predicted. Unfortunately, there are no universal principles for determining the ideal alteration; therefore, meticulous optimization is frequently required (8).

4.4 Macugen (Pegaptanib)

Currently, there is only one aptamer drug therapy that is approved, pegaptanib sodium, called **Macugen** (34). This drug is approved by the FDA and is one of the few therapies that exist to treat wet age-related macular degeneration (**AMD**), the other being the anticancer drug bevacizumab, a monoclonal antibody sold under the brand name **Avastin** from **Genentech**.

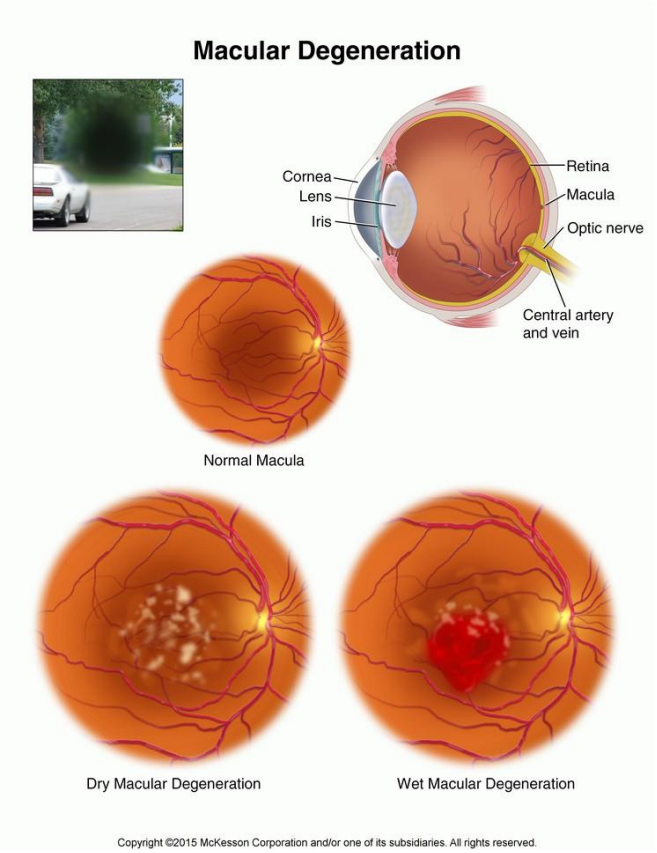

Fig. 16. Representation of the eye macula and both types of degeneration, dry on the left and wet on the right (35).

This disease is a progressive condition of macular degeneration that affects mainly older people. The macula is crucial for tasks such as reading, driving, and recognizing faces, among others (**Fig. 16**). **AMD** is one of the leading causes of blindness in people over the age of 50. Although this disease has been investigated for years, its exact cause is unknown (35).

The main triggers of this disease are old age, obesity, hypertension, smoking, and genetic predisposition. **AMD** affects the photoreceptors of the macula that are responsible for receiving light and transmitting signals to the brain to process the images. It usually affects both eyes, although the progression of the disease may differ between them. The most common symptoms are distorted vision, black spots in vision, and increased sensitivity to light. For its diagnosis, an exhaustive analysis of the meditative eye is done through different tests, such as optical tomography to evaluate the macular structure and fluorescent angiography to detect the growing capillaries (36).

The **Macugen** is focused on the Vascular endothelial growth factor A (**VEGF-A**) protein because this protein plays a crucial role in angiogenesis and cell proliferation. **VEGF-A** is primarily produced by stromal and tumor cells. This protein has the ability to stimulate the formation of new blood vessels by binding to receptors on endothelial cells lining the blood vessels. Wet **AMD** is more aggressive and rapid in causing vision loss compared to dry **AMD**. This is due to the abnormal growth of capillaries that leak and expel fluid and blood beneath the macula, causing damage to it and causing central vision to deteriorate rapidly (34).

The **VEGF 165** isoform was determined as the dominant. It is described as a heparin-binding, homodimeric 45-kDa glycoprotein that exists both in secreted and matrix-bound forms. Scientists at **NeXstar Pharmaceuticals** used three different iterations of the **SELEX** process to pick **VEGF165** as the target for the **anti-VEGF** aptamer that eventually became **Pegaptanib**. The initial study, published in 1994 (37), discovered aptamers that inhibited **VEGF's** effects in vitro, proving the potential use of an aptamer-based strategy for anti-angiogenesis.

The usage of **F-substituted** nucleotides to further improve affinity was described in 1995, after which it was reported that **NH_2-substituted** nucleotides were used to strengthen the resistance of **anti-VEGF** aptamers to nuclease attack (38). Three prospective **anti-VEGF** aptamers that were stable and high-affinity were characterized in 1998, and one of them was chosen for **pegaptanib's** development (34).

As mentioned before, the aptamer modifications lead to an increase in their resistance to nucleases and in vivo time. The **Table 4** shows the affinity and stability of three **anti-VEGF** aptamers. They were created by adding **2'-NH_2** pyrimidines and substituting **2'-OMe** for the **2'-hydroxyl** of purines. Two of them also have 3' and 5' phosphorothioate caps, and only one of them also has a **2'-OMe** purine substitution in the terminal of the caps. These few modifications could greatly increase in vivo pharmacokinetics.

Aptamer	Modification	Half-life in urine (hours)	Dissociation half-life	Binding affinity for VEGF (K_d, nM)	Binding affinity for PDGF (K_d, nM)	Ratio K_d PDGF/VEGF
NX-107	None (minimal ligand)	1.4	NR	NR	NR	NR
NX-178	3′ and 5′ caps	17	12 seconds	2.4	75	31
NX-213	3′ and 5′ caps + 2′-OMe purine substitution	131	8 minutes	0.14	91	650

Table 4. Modifications to three anti-VEGF aptamers and their pharmacokinetics (34).

To obtain what we now know as **Pegaptanib**, Ruckman et al. (39) used **2'-F**-pyrimidine-substituted **RNA** libraries during the **SELEX** process to generate anti-VEGF aptamers with higher affinities. This substitution promotes greater thermal stability of RNA-RNA, RNA-DNA, and DNA-DNA duplexes, which could lead to aptamers with higher affinities. In addition, oligonucleotides with **F-substituted** pyrimidines are more economical.

Recently, it was shown that an aptamer containing both **2'-F** and **2'-OMe** modifications was highly stable in vivo, persisting much longer in the circulation than an aptamer with only the **2'-OMe** modification.

After a few **SELEX** procedures, they found three families with higher affinities than the previous candidate aptamer. One candidate from each family was selected for an exhausting study, leading them to the three aptamers of the **Table 5**.

Aptamer	Length (nucleotides)	Binding affinity for VEGF (K_d, pM)	Dissociation half-life (seconds)	T_m (°C)	Binding dependent on divalent cations	$VEGF_{165}$ IC_{50} for VEGFR2 (M)	Miles assay (inhibition at 0.1 μM)
t22-OMe	23	72	60	49	No	$2\text{-}3 \times 10^{-12}$	13%
t2-OMe	29	130	170	66	No	6×10^{-11}	None
t44-OMe (pegaptanib)*	27	49	90	62	Yes	$2\text{-}3 \times 10^{-12}$	48%

Table 5. Addition of Ome modifications and their pharmacokinetics. Being t44-oMe the future Pegaptanib by the addition of PEG moiety (34).

The three selected aptamers demonstrated high affinity for **VEGF165** and **VEGF164**, but **t44-OMe** was the most effective at inhibiting vascular leakage from dermal microvessels following injection of **VEGF** into guinea pigs (34). The addition of a **5'**-linked 40-kDa polyethylene glycol moiety to **t44-OMe** improved inhibition to 83%, possibly due to prolonged tissue residence and plasma half-life. The **t44-OMe** polyethyleneglycol-linked aptamer was selected for further development and became **Pegaptanib** (**Fig. 17**).

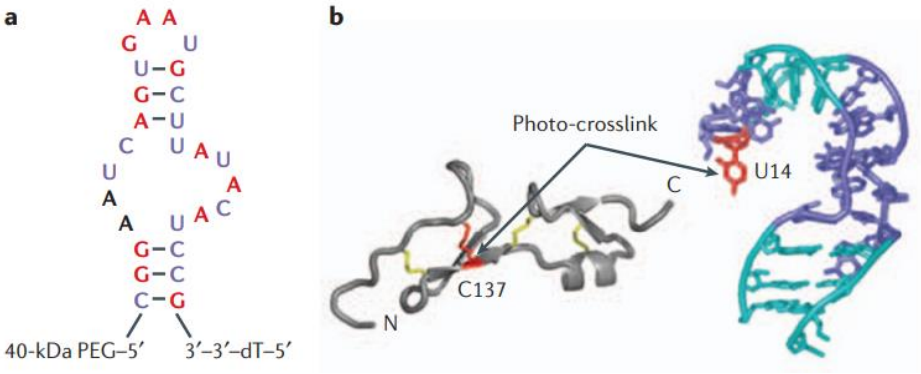

Fig. 17. Representation of Pegaptanib and target binding. (a) Secondary structure and sequence (34). Red bases are 2'-o-methylated purines, blue ones are 2'-fluorine pyrimidines, and black ones are unmodified. (b) Pegaptanib (blue) interaction with the heparin domain of VEGF165.

5. Conclusions

- Because of their unique capacity to detect and attach to specific molecules, aptamers have immense potential as therapeutic and diagnostic tools. Its use in electrochemical and optical detection can result in promising findings in early disease identification, monitoring therapy response, and assessing patient prognosis. As a result of their great selectivity and affinity, they are ideal options for targeting medical objectives and detecting biomarkers.
- Aptamers for therapeutic purposes are an attractive alternative to traditional antibodies. They can be useful for the development of personalized therapies due to their small size, stability, and ease of chemical manufacture. Additionally, its ability to inhibit or regulate the action of certain proteins opens possibilities for the treatment of a variety of diseases.
- The new SELEX methods outperform the standard method in terms of efficiency, quality, and diversity of aptamers obtained. These developments have strengthened the aptamer field and increased its potential in medicinal, diagnostic, and detection applications.
- Aptamer engineering, which includes the selection and optimization of existing aptamers in vitro, is a critical stage in their development as therapeutic and diagnostic tools. Improving their stability, selectivity, and affinity is crucial to ensuring clinical efficacy and economic viability.
- Due to their selectivity, sensitivity, speed, mobility, and interaction with current detection systems, aptasensors are ideal tools for detecting contaminants in food and water. By providing reliable and rapid identification of pollutants in these samples, these biosensors can play a critical role in maintaining food safety and preserving public health.
- Macugen is a novel and successful therapy option for AMD. Based on its high specificity, long-lasting effects, this therapy is an appealing choice for maintaining vision and improving patients' quality of life. Moreover, this is an excellent example of optimizing aptamers for increasing in vivo lifetime.

6. References

1. Ali MH, Elsherbiny ME, Emara M. Updates on aptamer research. Int J Mol Sci [Internet]. 2019;20(10):2511. Available from: http://dx.doi.org/10.3390/ijms20102511

2. Yang S, Li H, Xu L, Deng Z, Han W, Liu Y, et al. Oligonucleotide aptamer-mediated precision therapy of hematological malignancies. Mol Ther Nucleic Acids [Internet]. 2018;13:164–75. Available from: http://dx.doi.org/10.1016/j.omtn.2018.08.023

3. Ni S, Zhuo Z, Pan Y, Yu Y, Li F, Liu J, et al. Recent progress in aptamer discoveries and modifications for therapeutic applications. ACS Appl Mater Interfaces [Internet]. 2021;13(8):9500–19. Available from: http://dx.doi.org/10.1021/acsami.0c05750

4. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science [Internet]. 1990;249(4968):505–10. Available from: http://dx.doi.org/10.1126/science.2200121

5. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature [Internet]. 1990;346(6287):818–22. Available from: http://dx.doi.org/10.1038/346818a0

6. Wang T, Chen C, Larcher LM, Barrero RA, Veedu RN. Three decades of nucleic acid aptamer technologies: Lessons learned, progress and opportunities on aptamer development. Biotechnol Adv [Internet]. 2019;37(1):28–50. Available from: http://dx.doi.org/10.1016/j.biotechadv.2018.11.001

7. Stoltenburg R, Reinemann C, Strehlitz B. SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng [Internet]. 2007;24(4):381–403. Available from: http://dx.doi.org/10.1016/j.bioeng.2007.06.001

8. Zhuo Z, Yu Y, Wang M, Li J, Zhang Z, Liu J, et al. Recent advances in SELEX technology and aptamer applications in biomedicine. Int J Mol Sci [Internet]. 2017;18(10):2142. Available from: http://dx.doi.org/10.3390/ijms18102142

9. Mendonsa SD, Bowser MT. In vitro evolution of functional DNA using capillary electrophoresis. J Am Chem Soc [Internet]. 2004;126(1):20–1. Available from: http://dx.doi.org/10.1021/ja037832s

10. Eulberg D, Klussmann S. Spiegelmers: Biostable aptamers. ChemInform [Internet]. 2003;34(49). Available from: http://dx.doi.org/10.1002/chin.200349259

11. Kruspe S, Giangrande PH. Aptamer-siRNA chimeras: Discovery, progress, and future prospects. Biomedicines [Internet]. 2017;5(3):45. Available from: http://dx.doi.org/10.3390/biomedicines5030045

12. Strauss S, Nickels PC, Strauss MT, Jimenez Sabinina V, Ellenberg J, Carter JD, et al. Modified aptamers enable quantitative sub-10-nm cellular DNA-PAINT imaging. Nat Methods [Internet]. 2018;15(9):685–8. Available from: http://dx.doi.org/10.1038/s41592-018-0105-0

13. Pleiko K, Saulite L, Parfejevs V, Miculis K, Vjaters E, Riekstina U. Differential binding cell-SELEX method to identify cell-specific aptamers using high-throughput sequencing. Sci Rep [Internet]. 2019;9(1):8142. Available from: http://dx.doi.org/10.1038/s41598-019-44654-w

14. Kim D-H, Seo J-M, Shin K-J, Yang S-G. Design and clinical developments of aptamer-drug conjugates for targeted cancer therapy. Biomater Res [Internet]. 2021;25(1):42. Available from: http://dx.doi.org/10.1186/s40824-021-00244-4

15. Kohlberger M, Gadermaier G. SELEX: Critical factors and optimization strategies for successful aptamer selection. Biotechnol Appl Biochem [Internet]. 2022;69(5):1771–92. Available from: http://dx.doi.org/10.1002/bab.2244

16. Wang R, Zhu G, Mei L, Xie Y, Ma H, Ye M, et al. Automated modular synthesis of aptamer-drug conjugates for targeted drug delivery. J Am Chem Soc [Internet]. 2014;136(7):2731–4. Available from: http://dx.doi.org/10.1021/ja4117395

17. Soldevilla MM, Meraviglia-Crivelli de Caso D, Menon AP, Pastor F. Aptamer-iRNAs as therapeutics for cancer treatment. Pharmaceuticals (Basel) [Internet]. 2018;11(4):108. Available from: http://dx.doi.org/10.3390/ph11040108

18. Hori S-I, Herrera A, Rossi J, Zhou J. Current advances in aptamers for cancer diagnosis and therapy. Cancers (Basel) [Internet]. 2018;10(1):9. Available from: http://dx.doi.org/10.3390/cancers10010009

19. Keler T, He L, Ramakrishna V, Champion B. Antibody-targeted vaccines. Oncogene [Internet]. 2007;26(25):3758–67. Available from: http://dx.doi.org/10.1038/sj.onc.1210375

20. Luo Y-L, Shiao Y-S, Huang Y-F. Release of photoactivatable drugs from plasmonic nanoparticles for targeted cancer therapy. ACS Nano [Internet]. 2011;5(10):7796–804. Available from: http://dx.doi.org/10.1021/nn201592s

21. Song Y, Shi Y, Huang M, Wang W, Wang Y, Cheng J, et al. Bioinspired engineering of a multivalent aptamer-functionalized nanointerface to enhance the capture and release of circulating tumor cells. Angew Chem Int Ed Engl [Internet]. 2019;58(8):2236–40. Available from: http://dx.doi.org/10.1002/anie.201809337

22. Zhang K, Yue Y, Wu S, Liu W, Shi J, Zhang Z. Rapid capture and nondestructive release of extracellular vesicles using aptamer-based magnetic isolation. ACS Sens [Internet]. 2019;4(5):1245–51. Available from: http://dx.doi.org/10.1021/acssensors.9b00060

23. Sharma R, Ragavan KV, Thakur MS, Raghavarao KSMS. Recent advances in nanoparticle based aptasensors for food contaminants. Biosens Bioelectron [Internet]. 2015;74:612–27. Available from: http://dx.doi.org/10.1016/j.bios.2015.07.017

24. Li L, Li B, Qi Y, Jin Y. Label-free aptamer-based colorimetric detection of mercury ions in aqueous media using unmodified gold nanoparticles as colorimetric probe. Anal Bioanal Chem [Internet]. 2009;393(8):2051–7. Available from: http://dx.doi.org/10.1007/s00216-009-2640-0

25. Kimmel DW, LeBlanc G, Meschievitz ME, Cliffel DE. Electrochemical sensors and biosensors. Anal Chem [Internet]. 2012;84(2):685–707. Available from: http://dx.doi.org/10.1021/ac202878q

26. Zhou L, Wang J, Li D, Li Y. An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol A in milk samples. Food Chem [Internet]. 2014;162:34–40. Available from: http://dx.doi.org/10.1016/j.foodchem.2014.04.058

27. Kim YS, Kim JH, Kim IA, Lee SJ, Jurng J, Gu MB. A novel colorimetric aptasensor using gold nanoparticle for a highly sensitive and specific detection of oxytetracycline. Biosens Bioelectron [Internet]. 2010;26(4):1644–9. Available from: http://dx.doi.org/10.1016/j.bios.2010.08.046

28. Pang S, Labuza TP, He L. Development of a single aptamer-based surface enhanced Raman scattering method for rapid detection of multiple pesticides. Analyst [Internet]. 2014;139(8):1895–901. Available from: http://dx.doi.org/10.1039/c3an02263c

29. Song K-M, Cho M, Jo H, Min K, Jeon SH, Kim T, et al. Gold nanoparticle-based colorimetric detection of kanamycin using a DNA aptamer. Anal Biochem [Internet]. 2011;415(2):175–81. Available from: http://dx.doi.org/10.1016/j.ab.2011.04.007

30. Wu Y, Zhan S, Wang F, He L, Zhi W, Zhou P. Cationic polymers and aptamers mediated aggregation of gold nanoparticles for the colorimetric detection of arsenic(III) in aqueous solution. Chem Commun (Camb) [Internet]. 2012;48(37):4459–61. Available from: http://dx.doi.org/10.1039/c2cc30384a

31. Yuan J, Tao Z, Yu Y, Ma X, Xia Y, Wang L, et al. A visual detection method for Salmonella Typhimurium based on aptamer recognition and nanogold labeling. Food Control [Internet]. 2014;37:188–92. Available from: http://dx.doi.org/10.1016/j.foodcont.2013.09.046

32. Bruno JG. Application of DNA aptamers and quantum dots to lateral flow test strips for detection of foodborne pathogens with improved sensitivity versus colloidal gold. Pathogens [Internet]. 2014;3(2):341–55. Available from: http://dx.doi.org/10.3390/pathogens3020341

33. Sinha A, Gopinathan P, Chung Y-D, Lin H-Y, Li K-H, Ma H-P, et al. An integrated microfluidic platform to perform uninterrupted SELEX cycles to screen affinity reagents specific to cardiovascular biomarkers. Biosens Bioelectron [Internet]. 2018;122:104–12. Available from: http://dx.doi.org/10.1016/j.bios.2018.09.040

34. Ng EWM, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, Adamis AP. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov [Internet]. 2006;5(2):123–32. Available from: http://dx.doi.org/10.1038/nrd1955

35. Age-related macular degeneration [Internet]. Tufts Medical Center Community Care. 2017 [cited 2023 May 16]. Available from: https://hhma.org/healthadvisor/aha-macdeg-oph/

36. Michels S, Kurz-Levin M. Age-related macular degeneration (AMD). Ther Umsch [Internet]. 2009 [cited 2023 May 16];66(3):189–95. Available from: https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/age-related-macular-degeneration

37. Jellinek D, Green LS, Bell C, Janjić N. Inhibition of receptor binding by high-affinity RNA ligands to vascular endothelial growth factor. Biochemistry [Internet]. 1994;33(34):10450–6. Available from: http://dx.doi.org/10.1021/bi00200a028

38. Green LS, Jellinek D, Bell C, Beebe LA, Feistner BD, Gill SC, et al. Nuclease-resistant nucleic acid ligands to vascular permeability factor/vascular endothelial growth factor. Chem Biol [Internet]. 1995;2(10):683–95. Available from: http://dx.doi.org/10.1016/1074-5521(95)90032-2

39. Ruckman J, Green LS, Beeson J, Waugh S, Gillette WL, Henninger DD, et al. 2'-fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165): Inhibition of receptor binding and vegf-induced vascular permeability through interactions requiring the Exon 7-encoded domain. J Biol Chem [Internet]. 1998;273(32):20556–67. Available from: http://dx.doi.org/10.1074/jbc.273.32.20556